

NOVEL FIBROBLAST GROWTH FACTOR AND NUCLEIC ACIDS ENCODING SAME

RELATED APPLICATION

This application claims priority to United States Provisional patent application Serial
5 Number 60/145,899, entitled "Novel FGF-Like Protein," filed July 27, 1999. The contents of this priority application is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The invention generally relates to nucleic acids and polypeptides. The invention relates more particularly to nucleic acids encoding polypeptides related to a novel member of the
10 fibroblast growth factor family.

BACKGROUND OF THE INVENTION

The FGF family of proteins, whose prototypic members include acidic FGF (FGF-1) and basic FGF (FGF-2), bind to four related receptor tyrosine kinases. These FGF receptors are expressed on most types of cells in tissue culture. Dimerization of FGF receptor monomers upon
15 ligand binding has been reported to be a requisite for activation of the kinase domains, leading to receptor trans phosphorylation. FGF receptor-1 (FGFR-1), which shows the broadest expression pattern of the four FGF receptors, contains at least seven tyrosine phosphorylation sites. A number of signal transduction molecules are affected by binding with different affinities to these phosphorylation sites.

20 Expression of FGFs and their receptors in brains of perinatal and adult mice has been examined. Messenger RNA all FGF genes, with the exception of FGF-4, is detected in these tissues. FGF-3, FGF-6, FGF-7 and FGF-8 genes demonstrate higher expression in the late embryonic stages than in postnatal stages, suggesting that these members are involved in the late stages of brain development. In contrast, expression of FGF-1 and FGF-5 increased after birth. In
25 particular, FGF-6 expression in perinatal mice has been reported to be restricted to the central nervous system and skeletal muscles, with intense signals in the developing cerebrum in embryos

but in cerebellum in 5-day-old neonates. FGF-receptor (FGFR)-4, a cognate receptor for FGF-6, demonstrate similar spatiotemporal expression, suggesting that FGF-6 and FGFR-4 plays significant roles in the maturation of nervous system as a ligand-receptor system. According to Ozawa et al., these results strongly suggest that the various FGFs and their receptors are involved in the regulation of a variety of developmental processes of brain, such as proliferation and migration of neuronal progenitor cells, neuronal and glial differentiation, neurite extensions, and synapse formation.

Other members of the FGF polypeptide family include the FGF receptor tyrosine kinase (FGFR TK) family and the FGF receptor heparan sulfate proteoglycan (FGFR HS) family. These members interact to regulate active and specific FGFR signal transduction complexes. These regulatory activities are diversified throughout a broad range of organs and tissues, and in both normal and tumor tissues, in mammals. Regulated alternative messenger RNA (mRNA) splicing and combination of variant subdomains give rise to diversity of FGFR TK monomers. Divalent cations cooperate with the FGFR HS to conformationally restrict FGFR TK trans-phosphorylation, which causes depression of kinase activity and facilitates appropriate activation of the FGFR complex by FGF. For example, it is known that different point mutations in the FGFR TK commonly cause craniofacial and skeletal abnormalities of graded severity by graded increases in FGF-independent activity of total FGFR complexes. Other processes in which FGF family exerts important effects are liver growth and function and prostate tumor progression.

Glia-activating factor (GAF), another FGF family member, is a heparin-binding growth factor that was purified from the culture supernatant of a human glioma cell line. See, Miyamoto et al., 1993, *Mol Cell Biol* 13(7): 4251-4259. GAF shows a spectrum of activity slightly different from those of other known growth factors, and is designated as FGF-9. The human FGF-9 cDNA encodes a polypeptide of 208 amino acids. Sequence similarity to other members of the FGF family was estimated to be around 30%. Two cysteine residues and other consensus sequences found in other family members were also well conserved in the FGF-9 sequence. FGF-9 was found to have no typical signal sequence in its N terminus like those in acidic FGF and basic FGF. Acidic FGF and basic FGF are known not to be secreted from cells in a conventional manner. However, FGF-9 was found to be secreted efficiently from cDNA-transfected COS cells despite its lack of a typical signal sequence. It could be detected exclusively in the culture medium of cells. The secreted protein lacked no amino acid residues at

the N terminus with respect to those predicted by the cDNA sequence, except the initiation methionine. The rat FGF-9 cDNA was also cloned, and the structural analysis indicated that the FGF-9 gene is highly conserved.

SUMMARY OF THE INVENTION

5 The present invention is based, in part, upon the discovery of a nucleic acid encoding a novel polypeptide having homology to Fibroblast Growth Factor (FGF) protein. Fibroblast Grown Factor-CX (FGF-CX) polynucleotide sequences and the FGF-CX polypeptides encoded by these nucleic acid sequences, and fragments, homologs, analogs, and derivatives thereof, are claimed in the invention.

10 In one aspect, the invention provides an isolated FGF-CX nucleic acid (SEQ ID NO:1, as shown in FIG. 1), that encodes a FGF-CX polypeptide, or a fragment, homolog, analog or derivative thereof. The nucleic acid can include, *e.g.*, nucleic acid sequence encoding a polypeptide at least 85% identical to a polypeptide comprising the amino acid sequence of FIG. 1 (SEQ ID NO:2). The nucleic acid can be, *e.g.*, a genomic DNA fragment, or it can be a cDNA molecule.

15 Also included in the invention is a vector containing one or more of the nucleic acids described herein, and a cell containing the vectors or nucleic acids described herein.

 The present invention is also directed to host cells transformed with a recombinant expression vector comprising any of the nucleic acid molecules described above.

20 In one aspect, the invention includes a pharmaceutical composition that includes a FGF-CX nucleic acid and a pharmaceutically acceptable carrier or diluent. In a further aspect, the invention includes a substantially purified FGF-CX polypeptide, *e.g.*, any of the FGF-CX polypeptides encoded by a FGF-CX nucleic acid, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition that includes a FGF-CX polypeptide and a pharmaceutically acceptable carrier or diluent.

25 In a further aspect, the invention provides an antibody that binds specifically to a FGF-CX polypeptide. The antibody can be, *e.g.*, a monoclonal or polyclonal antibody, and fragments, homologs, analogs, and derivatives thereof. The invention also includes a pharmaceutical composition including FGF-CX antibody and a pharmaceutically acceptable carrier or diluent.

The present invention is also directed to isolated antibodies that bind to an epitope on a polypeptide encoded by any of the nucleic acid molecules described above.

The present invention is further directed to kits comprising antibodies that bind to a polypeptide encoded by any of the nucleic acid molecules described above and a negative control antibody.

The invention further provides a method for producing a FGF-CX polypeptide. The method includes providing a cell containing a FGF-CX nucleic acid, *e.g.*, a vector that includes a FGF-CX nucleic acid, and culturing the cell under conditions sufficient to express the FGF-CX polypeptide encoded by the nucleic acid. The expressed FGF-CX polypeptide is then recovered from the cell. Preferably, the cell produces little or no endogenous FGF-CX polypeptide. The cell can be, *e.g.*, a prokaryotic cell or eukaryotic cell.

The present invention provides a method of inducing an immune response in a mammal against a polypeptide encoded by any of the nucleic acid molecules disclosed above by administering to the mammal an amount of the polypeptide sufficient to induce the immune response.

The present invention is also directed to methods of identifying a compound that binds to FGF-CX polypeptide by contacting the FGF-CX polypeptide with a compound and determining whether the compound binds to the FGF-CX polypeptide.

The invention further provides methods of identifying a compound that modulates the activity of a FGF-CX polypeptide by contacting FGF-CX polypeptide with a compound and determining whether the FGF-CX polypeptide activity is modified.

The present invention is also directed to compounds that modulate FGF-CX polypeptide activity identified by contacting a FGF-CX polypeptide with the compound and determining whether the compound modifies activity of the FGF-CX polypeptide, binds to the FGF-CX polypeptide, or binds to a nucleic acid molecule encoding a FGF-CX polypeptide.

In another aspect, the invention provides a method of diagnosing a tissue proliferation-associated disorder, such as tumors, restenosis, psoriasis, diabetic and post-surgery complications, and rheumatoid arthritis, in a subject. The method includes providing a protein sample from the subject and measuring the amount of FGF-CX polypeptide in the subject sample. The amount of FGF-CX in the subject sample is then compared to the amount of FGF-CX polypeptide in a control protein sample. An alteration in the amount of FGF-CX polypeptide

in the subject protein sample relative to the amount of FGF-CX polypeptide in the control protein sample indicates the subject has a tissue proliferation-associated condition. A control sample is preferably taken from a matched individual, *i.e.*, an individual of similar age, sex, or other general condition but who is not suspected of having a tissue proliferation-associated condition. Alternatively, the control sample may be taken from the subject at a time when the subject is not suspected of having a tissue proliferation-associated disorder. In some embodiments, the FGF-CX polypeptide is detected using a FGF-CX antibody.

The invention is also directed to methods of inducing an immune response in a mammal against a polypeptide encoded by any of the nucleic acid molecules described above. The method includes administering to the mammal an amount of the polypeptide sufficient to induce the immune response.

In a further aspect, the invention includes a method of diagnosing a tissue proliferation-associated disorder, such as tumors, restenosis, psoriasis, diabetic and post-surgery complications, and rheumatoid arthritis, in a subject. The method includes providing a nucleic acid sample, *e.g.*, RNA or DNA, or both, from the subject and measuring the amount of the FGF-CX nucleic acid in the subject nucleic acid sample. The amount of FGF-CX nucleic acid sample in the subject nucleic acid is then compared to the amount of FGF-CX nucleic acid in a control sample. An alteration in the amount of FGF-CX nucleic acid in the sample relative to the amount of FGF-CX in the control sample indicates the subject has a tissue proliferation-associated disorder.

In a further aspect, the invention includes a method of diagnosing a tissue proliferation-associated disorder in a subject. The method includes providing a nucleic acid sample from the subject and identifying at least a portion of the nucleotide sequence of a FGF-CX nucleic acid in the subject nucleic acid sample. The FGF-CX nucleotide sequence of the subject sample is then compared to a FGF-CX nucleotide sequence of a control sample. An alteration in the FGF-CX nucleotide sequence in the sample relative to the FGF-CX nucleotide sequence in said control sample indicates the subject has a tissue proliferation-associated disorder.

In a still further aspect, the invention provides method of treating or preventing or delaying a tissue proliferation-associated disorder. The method includes administering to a subject in which such treatment or prevention or delay is desired a FGF-CX nucleic acid, a FGF-

CX polypeptide, or a FGF-CX antibody in an amount sufficient to treat, prevent, or delay a tissue proliferation-associated disorder in the subject.

The tissue proliferation-associated disorders diagnosed, treated, prevented or delayed using the FGF-CX nucleic acid molecules, polypeptides or antibodies can involve epithelial cells, *e.g.*, fibroblasts and keratinocytes in the anterior eye after surgery. Other tissue proliferation-associated disorder include, *e.g.*, tumors, restenosis, psoriasis, Dupuytren's contracture, diabetic complications, Kaposi sarcoma, and rheumatoid arthritis.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a representation of the nucleotide sequence (SEQ ID NO:1) and translated amino acid sequence (SEQ ID NO:2) of a novel FGF-CX polynucleotide and protein of the invention.

FIG. 2 is a BLASTN alignment of the nucleic acid sequence of SEQ ID NO:1 with a FGF-9-like Glia-Activating factor (GAF) sequence (SEQ ID NO:5).

FIG. 3 is a BLASTN alignment of the complementary strand of the nucleic acid sequence of SEQ ID NO:1 with three discontinuous segments (SEQ ID NOs:6-8 in panels A-C, respectively) of an extended genomic fragment of human chromosome 8 (GenBank accession number AB020858).

FIG. 4 is a graphic representation of a hydropathy plot of the FGF-CX polypeptide of SEQ ID NO:2, generated with a nineteen residue window.

FIG. 5 is a BLASTP alignment of the FGF-CX polypeptide sequence (SEQ ID NO:2) with a human FGF-9 (SEQ ID NO:9) indicating identical ("|") and positive ("+") residues.

FIG. 6 is a BLASTX alignment of the FGF-CX polypeptide sequence (SEQ ID NO:2) with murine FGF-9 (SEQ ID NO:10) indicating identical ("|") and positive ("+") residues.

5 FIG. 7 is a BLASTX alignment of the FGF-CX polypeptide sequence (SEQ ID NO:2) with rat FGF-9 (SEQ ID NO:11) indicating identical ("|") and positive ("+") residues.

FIG. 8 is a BLASTX alignment of the FGF-CX polypeptide sequence (SEQ ID NO:2) with *Xenopus* XFGF-20 (SEQ ID NO:12) indicating identical ("|") and positive ("+") residues.

10 FIG. 9 is a ClustalW Protein Sequence Alignment Analysis of four vertebrate FGF-like proteins (SEQ ID NO:9-12) with the FGF-CX protein (SEQ ID NO:2) of the present invention. Black, gray and white represent identical, conserved and nonconserved residues in the alignment, respectively.

DETAILED DESCRIPTION OF THE INVENTION

15 The invention is based in part on the discovery of novel FGF-CX nucleic acid sequence, which encode a polypeptide that is a member of the fibroblast growth factor family.

Included within the invention are FGF-CX nucleic acids, isolated nucleic acids that encode FGF-CX polypeptide or a portion thereof, FGF-CX polypeptides, vectors containing these nucleic acids, host cells transformed with the FGF-CX nucleic acids, anti-FGF-CX antibodies, and pharmaceutical compositions. Also disclosed are methods of making FGF-CX polypeptides, as well as methods of screening, diagnosing, treating conditions using these compounds, and methods of screening compounds that modulate FGF-CX polypeptide activity. 20 Table 1 below delineates the sequence descriptors that are used throughout the invention.

TABLE 1

SEQ ID NO	SEQUENCE DESCRIPTOR
1	Human FGF-CX nucleotide sequence
2	Human FGF-CX polypeptide sequence
3	FGF-CX forward primer
4	FGF-CX reverse primer
5	Glia Activating Factor (GAF)
6	Human genomic fragment - bp 15927-16214
7	Human genomic fragment - bp 7257-7511
8	Human genomic fragment - bp 9837-9942
9	Human FGF-9

- 10 Mouse FGF-9
- 11 Rat FGF-9
- 12 Xenopus FGF-20
- 13 Human FGF-CX hydrophobic domain (aa 90-115)

The FGF-CX nucleic acids and polypeptides, as well as FGF-CX antibodies, as well as pharmaceutical compositions discussed herein, are useful, *inter alia*, in treating tissue proliferation-associated disorders. These tissue proliferation-associated disorders can include epithelial cells, *e.g.*, fibroblasts and keratinocytes in the anterior eye after surgery. Other tissue proliferation-associated disorder include, *e.g.*, tumors, restenosis, psoriasis, Dupuytren's contracture, diabetic complications, Kaposi sarcoma, and rheumatoid arthritis.

The present invention discloses a nucleotide sequence encoding a novel fibroblast growth factor designated fibroblast growth factor-CX (FGF-CX) herein (see FIG. 1; SEQ ID NO:1).

10 This coding sequence was identified in human genomic DNA sequences. The DNA sequence has 633 bases that encode a polypeptide predicted to have 211 amino acid residues. The predicted molecular weight of FGF-CX, based on the sequence shown in FIG. 1 and SEQ ID NO:2, is 23498.4 Da.

15 The FGF-CX nucleic acid sequence was used in a BLASTN search. The FGF-CX nucleotide sequence has a high similarity to murine fibroblast growth factor 9 (FGF-9) (392 of 543 bases identical, or 72%; GenBank accession number S82023) and to human DNA encoding glia activating factor (GAP) (385 of 554 bases identical, or 69%; GenBank accession number E05822, also termed FGF-9). In addition, FGF-CX was found to have a comparable degree of identity (311 of 424 bases identical, or 73%) to a GAP sequence (SEQ ID NO:5) disclosed by

20 Naruo *et al.* in Japanese Patent: JP 1993301893 entitled "Glia-Activating Factor And Its Production" (see FIG. 2).

An additional BLASTN search, shown in FIG. 3A-C, identified a human genomic fragment of approximately 100,000 bp from chromosome 8 (GenBank accession number AB020858, Homo sapiens genomic DNA of chromosome 8p21.3-p22) with 3 widely separated

25 sequences that, each individually, match a different portion of the contiguous sequence of the present invention almost perfectly. Specifically, the chromosomal sequence from bp 15927-16214 (SEQ ID NO: 6) shown in FIG. 3A is 99% identical to the noncoding FGF-CX strand from bp 1-289. The chromosomal sequence from bp 7257-7511 (SEQ ID NO: 7) shown in FIG.

3B is 98% identical to the noncoding FGF-CX strand from bp 380-633. The chromosomal sequence from bp 9837-9942 (SEQ ID NO: 8) shown in FIG. 3C is 100% identical to the noncoding FGF-CX strand from bp 286-391. However, these genomic fragments shown in FIG. 3A-C differ from the present invention in that the genomic fragments (a) are separated from one another by several thousand bases, (b) have a different order in the genomic sequence compared to the order of the equivalent sequences in the presently disclosed nucleotide sequence, (c) represent the noncoding strand of the disclosed invention, which is indicated in FIG. 3 by the reversed numbering of the FGF-CX nucleotides, and (d) are not associated or identified in the GenBank disclosure in any way. Finally, even if the proper coding strand of the genomic sequence shown in FIG. 3A had previously been identified, which it had not, and that strand had been identified as having an open reading frame, which it had not, this portion of the genomic sequence has a one base deletion compared to the FGF-CX sequence of SEQ ID NO:1. See, FIG. 3A. Therefore, the predicted polypeptide from the genomic sequence would be a different, frame-shifted, polypeptide from that point on compared to SEQ ID NO:2.

The polypeptide sequence in FIG. 1 (SEQ ID NO:2) is predicted by the program PSORT to have high probabilities for sorting through the membrane of the endoplasmic reticulum and of the microbody (peroxisome). In addition, although it does not have a predicted cleavable signal sequence at its N-terminus, the hydropathy plot in FIG. 4 shows FGF-CX has a prominent hydrophobic segment at amino acid positions about 90 to about 115 (SEQ ID NO:13). This single hydrophobic region is known to be a sorting signal in other members of the FGF family. According, a polypeptide that includes the amino acids of SEQ ID NO:13 is useful as a sorting signal, allowing secretion through various cellular membranes, such as the endoplasmic reticulum, the Golgi membrane or the plasma membrane.

A BLASTP alignment of the first 208 amino acids of the FGF-CX polypeptide sequence (SEQ ID NO:2) with a human FGF-9 (SEQ ID NO:9) is shown in FIG. 5. See, SwissProt accession number P31371 for Glia-Activating Factor Precursor (GAF) (Fibroblast Growth Factor-9); Miyamoto *et al.* 1993 *Mol. Cell. Biol.* 13:4251-4259; and Naruo *et al.* 1993 *J. Biol. Chem.* 268:2857-2864. BLASTX alignments of the first 208 amino acids of the FGF-CX polypeptide (SEQ ID NO:2, translated from SEQ ID NO:1) with the mouse FGF-9 (SEQ ID NO:10) and rat FGF-9 (SEQ ID NO:11) sequences are shown in FIGS. 6 and 7, respectively. See, SwissProt accession number P54130 for Glia-Activating Factor Precursor (GAF)

(Fibroblast Growth Factor-9), Santos-Ocampo *et al.*, 1996 *J. Biol. Chem.* 271:1726-1731, for mouse FGF-9; and SwissProt accession number P36364 Glia-Activating Factor Precursor (GAF) (Fibroblast Growth Factor-9) (FGF-9), Miyamoto, 1993 *Mol. Cell. Biol.* 13:4251-4259, for rat FGF-9. As indicated by the bars ("|") in FIGS. 5-7, FGF-9 sequences of all three species have
 5 147 of 208 residues identical with FGF-CX (SEQ ID NO:2), for an overall sequence identity of 70%. In addition, 170 of 208 residues are positive to the sequence of FGF-CX (SEQ ID NO:2), for an overall percentage of positive residues of 81%. Positive residues include those residues that are either identical ("|") or have a conservative amino acid substitution ("+") in the same relative position of the compared sequences when aligned, see below.

10 The full length FGF-CX polypeptide (SEQ ID NO:2) was also aligned by BLASTX with *Xenopus* XFGE-20 (SEQ ID NO:12). As shown in FIG. 8, FGF-CX has 170 of 211 (80%) identical residues, and 189 of 211 (89%) positive residues compared with *Xenopus* XFGE-20. *Xenopus* XFGE-20 was obtained recently from a cDNA library prepared at the tailbud stage using the product of degenerate PCR performed with primers based on mammalian FGF-9s as a
 15 probe. See, Koga *et al.*, 1999 *Biochem Biophys Res Commun* 261(3):756-765. The deduced 208 amino acid sequence of the XFGE-20 open reading frame contains a motif characteristic of the FGF family. XFGE-20 has a 73.1% overall similarity to XFGE-9 but differs from XFGE-9 in its amino-terminal region (33.3% similarity). This resembles the similarity seen for the presently disclosed SEQ ID NO:2 with respect to various mammalian FGF-9 sequences, including human
 20 (see above). See, FIGS. 6-9.

XFGE-20 and XFGE-9 expression is distinct from each other. XFGE-20 mRNA is expressed in diploid cells, in embryos at and after the blastula stage, and specifically in the stomach and testis of adults; whereas XFGE-9 mRNA is expressed maternally in eggs and in many adult tissues. Koga *et al.*, above. Correct expression of XFGE-20 during gastrulation
 25 appears to be required for the formation of normal head structures in *Xenopus laevis*. When XFGE-20 mRNA was overexpressed in early embryos, gastrulation was abnormal and development of anterior structures was suppressed. See, Koga *et al.*, above. In such embryos, expression of the *Xbra* transcript, among those tested, was suppressed during gastrulation, indicating that expression of the *Xbra* gene mediates XFGE-20 effects. See, Koga *et al.*, above.

30 The present inventors believe that the results and observations provided by Koga *et al.*, namely, the general correlation of expression of the gene in proliferating tissues including ova,

testis, stomach, and many tissues in the maternal frog, suggests a role for XFGF-20 in the maintenance of tissues that normally undergo regeneration in a functioning organism.

A ClustalW multiple protein alignment for the five vertebrate FGF-like proteins discussed above, including the FGF-CX of the present invention, is shown in FIG. 9. The three mammalian proteins (SEQ ID NOs:9-11) resemble each other very closely but differ considerably from the FGF-CX protein of the present invention (SEQ ID NO:2). Also, the *Xenopus* XFGF-20 (SEQ ID NO:12) and the sequence of SEQ ID NO:2 resemble each other more closely than those of FGF-9.

FGF-CX Nucleic Acids

The novel nucleic acids of the invention include those that encode a FGF-CX or FGF-CX-like protein. Among these nucleic acids is the nucleic acid whose sequence is provided in FIG. 1 and SEQ ID NO:1, or a fragment thereof. Additionally, the invention includes mutant or variant nucleic acids of SEQ ID NO:1, or a fragment thereof, any of whose bases may be changed from the corresponding base shown in FIG. 1 while still encoding a protein that maintains its FGF-CX-like activities and physiological functions. The invention further includes the complement of the nucleic acid sequence of SEQ ID NO:1, including fragments, derivatives, analogs and homolog thereof. Examples of the complementary strand of portions of FGF-CX are shown in FIG. 3. The invention additionally includes nucleic acids or nucleic acid fragments, or complements thereto, whose structures include chemical modifications.

One aspect of the invention pertains to isolated nucleic acid molecules that encode FGF-CX proteins or biologically active portions thereof. Also included are nucleic acid fragments sufficient for use as hybridization probes to identify FGF-CX-encoding nucleic acids (*e.g.*, FGF-CX mRNA) and fragments for use as polymerase chain reaction (PCR) primers for the amplification or mutation of FGF-CX nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

"Probes" refer to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt), 100 nt, or as many as about, *e.g.*, 6,000 nt, depending on use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer

length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

5 An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules that are present in the natural source of the nucleic acid. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules. Preferably,
10 an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated FGF-CX nucleic acid molecule can contain less than about 50 kb, 25 kb, 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in
15 genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the
20 nucleotide sequence of SEQ ID NO:1, or a complement of any of this nucleotide sequence, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequence of SEQ ID NO:1 as a hybridization probe, FGF-CX nucleic acid sequences can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, eds., MOLECULAR CLONING: A LABORATORY
25 MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, eds., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR
30 amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to

FGF-CX nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction.

5 A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of a nucleic acid sequence having about 10 nt, 50 nt, or 100 nt in length, preferably about 15 nt to 30 nt in length. In one embodiment, an oligonucleotide comprising a nucleic acid molecule
10 less than 100 nt in length would further comprise at least 6 contiguous nucleotides of SEQ ID NO:1, or a complement thereof. Oligonucleotides may be chemically synthesized and may be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1.

15 In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in SEQ ID NO:1, or a portion of this nucleotide sequence. A nucleic acid molecule that is complementary to the nucleotide sequence shown in SEQ ID NO:1 is one that is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1 that it can hydrogen bond with little or no
20 mismatches to the nucleotide sequence shown in SEQ ID NO:1, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, Von der
25 Waals, hydrophobic interactions, etc. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

30 Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of SEQ ID NO:1, *e.g.*, a fragment that can be used as a probe or primer, or

a fragment encoding a biologically active portion of FGF-CX. Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 70%, 80%, 85%, 90%, 95%, 98%, or even 99% identity (with a preferred identity of 80-99%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below. An exemplary program is the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison, WI) using the default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2: 482-489, which is incorporated herein by reference in its entirety).

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of FGF-CX polypeptide. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively,

isoforms can be encoded by different genes. In the present invention, homologous nucleotide sequences include nucleotide sequences encoding for a FGF-CX polypeptide of species other than humans, including, but not limited to, mammals, and thus can include, *e.g.*, mouse, rat, rabbit, dog, cat, cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding human FGF-CX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in SEQ ID NO:2, as well as a polypeptide having FGF-CX activity. Biological activities of the FGF-CX proteins are described below. A homologous amino acid sequence does not encode the amino acid sequence of a human FGF-CX polypeptide.

The nucleotide sequence determined from the cloning of the human FGF-CX gene allows for the generation of probes and primers designed for use in identifying and/or cloning FGF-CX homologues in other cell types, *e.g.*, from other tissues, as well as FGF-CX homologues from other mammals. The probe/primer typically comprises a substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 or more consecutive sense strand nucleotide sequence of SEQ ID NO:1; or an anti-sense strand nucleotide sequence of SEQ ID NO:1; or of a naturally occurring mutant of SEQ ID NO:1.

Probes based on the human FGF-CX nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a FGF-CX protein, such as by measuring a level of a FGF-CX-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting FGF-CX mRNA levels or determining whether a genomic FGF-CX gene has been mutated or deleted.

"A polypeptide having a biologically active portion of FGF-CX" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically active portion of

FGF-CX" can be prepared by isolating a portion of SEQ ID NO:1, that encodes a polypeptide having a FGF-CX biological activity (biological activities of the FGF-CX proteins are described below), expressing the encoded portion of FGF-CX protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of FGF-CX. For example, a nucleic acid fragment encoding a biologically active portion of FGF-CX can optionally include an ATP-binding domain. In another embodiment, a nucleic acid fragment encoding a biologically active portion of FGF-CX includes one or more regions.

FGF-CX variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequences shown in Fig.1 due to degeneracy of the genetic code. These nucleic acids thus encode the same FGF-CX protein as that encoded by the nucleotide sequence shown in SEQ ID NO:1. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence shown in SEQ ID NO:2.

In addition to the human FGF-CX nucleotide sequence shown in SEQ ID NO:1, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of FGF-CX may exist within a population (e.g., the human population). Such genetic polymorphism in the FGF-CX gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a FGF-CX protein, preferably a mammalian FGF-CX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the FGF-CX gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in FGF-CX that are the result of natural allelic variation and that do not alter the functional activity of FGF-CX are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding FGF-CX proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of SEQ ID NO:1, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the FGF-CX cDNAs of the invention can be isolated based on their homology to the human FGF-CX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a soluble human FGF-CX

cDNA can be isolated based on its homology to human membrane-bound FGF-CX. Likewise, a membrane-bound human FGF-CX cDNA can be isolated based on its homology to soluble human FGF-CX.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250, 500 or 750 nucleotides in length. In another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding FGF-CX proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at T_m , 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (*e.g.*, 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions is hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C. This hybridization is followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 corresponds to a naturally occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A

LABORATORY MANUAL, Stockton Press, NY; Shilo and Weinberg, 1981, *Proc Natl Acad Sci USA* 78: 6789-6792.

Conservative mutations

5 In addition to naturally-occurring allelic variants of the FGF-CX sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO:1, thereby leading to changes in the amino acid sequence of the encoded FGF-CX protein, without altering the functional ability of the FGF-CX protein. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1. A

10 "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of FGF-CX without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the FGF-CX proteins of the present invention, are predicted to be particularly unamenable to alteration.

15 In addition, amino acid residues that are conserved among FGF family members, as indicated by the alignment presented as FIG. 9, are predicted to be less amenable to alteration. For example, FGF-CX proteins of the present invention can contain at least one domain that is a typically conserved region in FGF family members, *i.e.*, FGF-9 and XFGE-20 proteins, and FGF-CX homologs. As such, these conserved domains are not likely to be amenable to mutation.

20 Other amino acid residues, however, (*e.g.*, those that are not conserved or only semi-conserved among members of the FGF proteins) may not be as essential for activity and thus are more likely to be amenable to alteration.

Another aspect of the invention pertains to nucleic acid molecules encoding FGF-CX proteins that contain changes in amino acid residues that are not essential for activity. Such

25 FGF-CX proteins differ in amino acid sequence from SEQ ID NO:2, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 75% homologous to the amino acid sequence of SEQ ID NO:2. Preferably, the protein encoded by the nucleic acid is at least about 80% homologous to SEQ ID NO:2, more preferably at least

30 about 90%, 95%, 98%, and most preferably at least about 99% homologous to SEQ ID NO:2.

An isolated nucleic acid molecule encoding a FGF-CX protein homologous to the protein of SEQ ID NO:2 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

5 Mutations can be introduced into SEQ ID NO:1 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar
10 side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and
15 aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in FGF-CX is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a FGF-CX coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for FGF-CX biological activity to identify mutants that
20 retain activity. Following mutagenesis of SEQ ID NO:1, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

In one embodiment, a mutant FGF-CX protein can be assayed for (1) the ability to form protein:protein interactions with other FGF-CX proteins, other cell-surface proteins, or
25 biologically active portions thereof, (2) complex formation between a mutant FGF-CX protein and a FGF-CX receptor; (3) the ability of a mutant FGF-CX protein to bind to an intracellular target protein or biologically active portion thereof; (*e.g.*, avidin proteins); (4) the ability to bind BRA protein; or (5) the ability to specifically bind an anti-FGF-CX protein antibody.

Antisense

30 Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide

sequence of SEQ ID NO:1, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire FGF-CX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a FGF-CX protein of SEQ ID NO:2 or antisense nucleic acids complementary to a FGF-CX nucleic acid sequence of SEQ ID NO:1 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding FGF-CX. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues (*e.g.*, the protein coding region of human FGF-CX corresponds to SEQ ID NO:2). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding FGF-CX. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding FGF-CX disclosed herein (*e.g.*, SEQ ID NO:1), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of FGF-CX mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of FGF-CX mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of FGF-CX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a FGF-CX protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules,

vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

Ribozymes and PNA moieties

Such modifications include, by way of nonlimiting example, modified bases, and nucleic acids whose sugar phosphate backbones are modified or derivatized. These modifications are carried out at least in part to enhance the chemical stability of the modified nucleic acid, such that they may be used, for example, as antisense binding nucleic acids in therapeutic applications in a subject.

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave FGF-CX mRNA transcripts to thereby inhibit translation of FGF-CX mRNA. A ribozyme having specificity for a FGF-CX-encoding nucleic acid can be designed based upon the nucleotide sequence of a FGF-CX DNA disclosed herein (*i.e.*, SEQ ID NO:1). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a FGF-CX-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742. Alternatively, FGF-CX mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, FGF-CX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the FGF-CX (*e.g.*, the FGF-CX promoter and/or enhancers) to form triple helical structures that prevent transcription of the FGF-CX gene

in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of FGF-CX can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

PNAs of FGF-CX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of FGF-CX can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

In another embodiment, PNAs of FGF-CX can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of FGF-CX can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid

support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

FGF-CX polypeptides

The novel protein of the invention includes the FGF-CX-like protein whose sequence is provided in FIG. 1 (SEQ ID NO:2). The invention also includes a mutant or variant protein any of whose residues may be changed from the corresponding residue shown in FIG. 1 while still encoding a protein that maintains its FGF-CX-like activities and physiological functions, or a functional fragment thereof. In the mutant or variant protein, up to 20% or more of the residues may be so changed.

In general, an FGF-CX-like variant that preserves FGF-CX-like function includes any variant in which residues at a particular position in the sequence have been substituted by other amino acids, and further include the possibility of inserting an additional residue or residues between two residues of the parent protein as well as the possibility of deleting one or more residues from the parent sequence. Any amino acid substitution, insertion, or deletion is encompassed by the invention. In favorable circumstances, the substitution is a conservative

substitution as defined above. Furthermore, without limiting the scope of the invention, the following positions in Table 2 (using the numbering provided in SEQ ID NO:2) may be substituted as indicated, such that a mutant or variant protein may include one or more than one of the substitutions indicated. The suggested substitutions do not limit the range of possible substitutions that may be made at a given position.

TABLE 2.

	Position	Possible Substitution
	6:	Glu to Asp
	9:	Gly to Ser, Thr, or Asn
10	10:	Phe to Tyr
	11:	Leu to Phe or Ile
	15:	Glu to Asp
	16:	Gly to Ala
	17:	Leu to Ile or Val
15	19:	Gln may be deleted
	21:	Val to Phe or Ile
	31:	Gly to Lys, Arg, Ser, or Ala
	33:	Arg to Lys or Ser
	35:	Pro to Leu or Val
20	38:	Gly to Asn or Ser
	39:	Glu to Asp
	40:	Arg to Lys, His, or Pro
	42:	Ser to Thr, Ala, or Gly
	43:	Ala to Gln, Asn, or Ser
25	48:	Ala to Ser or Gly
	51:	Gly to Ala
	53:	Gly to Ala or deleted
	54:	Ala to Gly, Val, or deleted
	55:	Ala to Ser or Thr
30	56:	Gln to Asp, Glu, or Asn
	58:	Ala to Ser, Thr, Asn, Gln, Asp, or Glu
	61:	His to Gln, Asn, Lys, or Arg
	78:	Gln to Asn, Glu, or Asp
	80:	Leu to Phe or Ile
35	82:	Asp to Glu, Asn, or Gln
	84:	Ser to Asn, Thr, or Gln
	85:	Val to Ile
	90:	Gln to Asn or Lys
	103:	Val to Ile
40	115:	Ser to Thr
	123:	Asp to Glu
	128:	Tyr to Phe
	135:	Ser to Thr, Gln, or Asn

	138:	Ile to Val or Leu
	155:	Ile to Leu
	159:	Gly to Val or Ala
	161:	Thr to Ser
5	166:	Phe to Tyr
	177:	Asp to Glu
	181:	Ser to Ala or Thr
	198:	Glu to Asp
	199:	Arg to Lys
10	207:	Leu to Ile or Val
	209:	Met to any residue
	211:	Thr to Ser

One aspect of the invention pertains to isolated FGF-CX proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-FGF-CX antibodies. In one embodiment, native FGF-CX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, FGF-CX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a FGF-CX protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the FGF-CX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of FGF-CX protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of FGF-CX protein having less than about 30% (by dry weight) of non-FGF-CX protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-FGF-CX protein, still more preferably less than about 10% of non-FGF-CX protein, and most preferably less than about 5% non-FGF-CX protein. When the FGF-CX protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of FGF-CX protein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of FGF-CX protein having less than about 30% (by dry weight) of chemical precursors or non-FGF-CX chemicals, more preferably less than about 20% chemical precursors or non-FGF-CX chemicals, still more preferably less than about 10% chemical precursors or non-FGF-CX chemicals, and most preferably less than about 5% chemical precursors or non-FGF-CX chemicals.

Biologically active portions of a FGF-CX protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the FGF-CX protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2 that include fewer amino acids than the full length FGF-CX proteins, and exhibit at least one activity of a FGF-CX protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the FGF-CX protein. A biologically active portion of a FGF-CX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

A biologically active portion of a FGF-CX protein of the present invention may contain at least one of the above-identified domains conserved between the FGF family of proteins. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native FGF-CX protein.

In an embodiment, the FGF-CX protein has an amino acid sequence shown in SEQ ID NO:2. In other embodiments, the FGF-CX protein is substantially homologous to SEQ ID NO:2 and retains the functional activity of the protein of SEQ ID NO:2, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail below. Accordingly, in another embodiment, the FGF-CX protein is a protein that comprises an amino acid sequence at least about 45% homologous to the amino acid sequence of SEQ ID NO:2 and retains the functional activity of the FGF-CX proteins of SEQ ID NO:2.

Determining homology between two or more sequences

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in either

of the sequences being compared for optimal alignment between the sequences). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. See, *Needleman and Wunsch* 1970 *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding) part of the DNA sequence shown in SEQ ID NO:1.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region. The term "percentage of positive residues" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical and conservative amino acid substitutions, as defined above, occur in both sequences to yield the number of matched positions, dividing the number of matched positions

by the total number of positions in the region of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of positive residues.

Chimeric and fusion proteins

The invention also provides FGF-CX chimeric or fusion proteins. As used herein, a FGF-CX "chimeric protein" or "fusion protein" comprises a FGF-CX polypeptide operatively
5 linked to a non-FGF-CX polypeptide. A "FGF-CX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to FGF-CX, whereas a "non-FGF-CX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the FGF-CX protein, *e.g.*, a protein that is different from the FGF-
10 CX protein and that is derived from the same or a different organism. Within a FGF-CX fusion protein the FGF-CX polypeptide can correspond to all or a portion of a FGF-CX protein. In one embodiment, a FGF-CX fusion protein comprises at least one biologically active portion of a FGF-CX protein. In another embodiment, a FGF-CX fusion protein comprises at least two biologically active portions of a FGF-CX protein. Within the fusion protein, the term
15 "operatively linked" is intended to indicate that the FGF-CX polypeptide and the non-FGF-CX polypeptide are fused in-frame to each other. The non-FGF-CX polypeptide can be fused to the N-terminus or C-terminus of the FGF-CX polypeptide.

For example, in one embodiment a FGF-CX fusion protein comprises a FGF-CX polypeptide operably linked to the extracellular domain of a second protein. Such fusion
20 proteins can be further utilized in screening assays for compounds that modulate FGF-CX activity (such assays are described in detail below).

In another embodiment, the fusion protein is a GST-FGF-CX fusion protein in which the FGF-CX sequences are fused to the C-terminus of the GST (*i.e.*, glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant FGF-CX.

25 In yet another embodiment, the fusion protein is a FGF-CX protein containing a heterologous signal sequence at its N-terminus. For example, the native FGF-CX signal sequence (*i.e.*, amino acids 1 to 20 of SEQ ID NO:2) can be removed and replaced with a signal sequence from another protein. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of FGF-CX can be increased through use of a heterologous signal sequence.

30 In another embodiment, the fusion protein is a FGF-CX-immunoglobulin fusion protein in which the FGF-CX sequences comprising one or more domains are fused to sequences derived

from a member of the immunoglobulin protein family. The FGF-CX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a FGF-CX ligand and a FGF-CX protein on the surface of a cell, to thereby suppress FGF-CX-mediated signal transduction *in vivo*. In one nonlimiting example, a contemplated FGF-CX ligand of the invention is the FGF-CX receptor. The FGF-CX-immunoglobulin fusion proteins can be used to affect the bioavailability of a FGF-CX cognate ligand. Inhibition of the FGF-CX ligand/FGF-CX interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, as well as modulating (*e.g.*, promoting or inhibiting) cell survival. Moreover, the FGF-CX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-FGF-CX antibodies in a subject, to purify FGF-CX ligands, and in screening assays to identify molecules that inhibit the interaction of FGF-CX with a FGF-CX ligand.

A FGF-CX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel et al. (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A FGF-CX-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the FGF-CX protein.

FGF-CX agonists and antagonists

The present invention also pertains to variants of the FGF-CX proteins that function as either FGF-CX agonists (mimetics) or as FGF-CX antagonists. Variants of the FGF-CX protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of the FGF-CX

protein. An agonist of the FGF-CX protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the FGF-CX protein. An antagonist of the FGF-CX protein can inhibit one or more of the activities of the naturally occurring form of the FGF-CX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the FGF-CX protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the FGF-CX proteins.

10 Variants of the FGF-CX protein that function as either FGF-CX agonists (mimetics) or as FGF-CX antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the FGF-CX protein for FGF-CX protein agonist or antagonist activity. In one embodiment, a variegated library of FGF-CX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of FGF-CX variants can be produced by, for example, enzymatically ligating a mixture of
15 synthetic oligonucleotides into gene sequences such that a degenerate set of potential FGF-CX sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of FGF-CX sequences therein. There are a variety of methods which can be used to produce libraries of potential FGF-CX variants from a
20 degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential FGF-CX sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang
25 (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu Rev Biochem* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucl Acid Res* 11:477.

Polypeptide libraries

In addition, libraries of fragments of the FGF-CX protein coding sequence can be used to generate a variegated population of FGF-CX fragments for screening and subsequent selection of
30 variants of a FGF-CX protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a FGF-CX coding sequence with a

nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the FGF-CX protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of gene libraries generated by the combinatorial mutagenesis of FGF-CX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify FGF-CX variants (Arkin and Yourvan (1992) PNAS 89:7811-7815; Delgrave *et al.* (1993) Protein Engineering 6:327-331).

Anti-FGF-CX Antibodies

The invention further encompasses antibodies and antibody fragments, such as F_{ab} or $(F_{ab})_2$, that bind immunospecifically to any of the proteins of the invention.

An isolated FGF-CX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind FGF-CX using standard techniques for polyclonal and monoclonal antibody preparation. Full-length FGF-CX protein can be used. Alternatively, the invention provides antigenic peptide fragments of FGF-CX for use as immunogens. The antigenic peptide of FGF-CX comprises at least 4 amino acid residues of the amino acid sequence shown in SEQ ID NO:2. The antigenic peptide encompasses an epitope of FGF-CX such that an antibody raised against the peptide forms a specific immune complex with FGF-CX. The antigenic peptide may comprise at least 6 aa residues, at least 8 aa residues, at least 10 aa residues, at least 15 aa residues, at least 20 aa residues, or at least 30 aa residues. In one

embodiment of the invention, the antigenic peptide comprises a polypeptide comprising at least 6 contiguous amino acids of SEQ ID NO:2.

In an embodiment of the invention, epitopes encompassed by the antigenic peptide are regions of FGF-CX that are located on the surface of the protein, *e.g.*, hydrophilic regions. A hydrophobicity analysis of the human FGF-CX protein sequence, shown in FIG. 4, indicates that the regions between amino acids 30-50 and amino acids 120-195 are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, Proc. Nat. Acad. Sci. USA 78: 3824-3828; Kyte and Doolittle 1982, J. Mol. Biol. 157: 105-142, each incorporated herein by reference in their entirety.

As disclosed herein, FGF-CX protein sequence of SEQ ID NO:2, or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen, such as FGF-CX. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and $F_{(ab')_2}$ fragments, and an F_{ab} expression library. In a specific embodiment, antibodies to human FGF-CX proteins are disclosed. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to a FGF-CX protein sequence of SEQ ID NO:2 or derivative, fragment, analog or homolog thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly expressed FGF-CX protein or a chemically synthesized FGF-CX polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances

(e.g., lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against FGF-CX can be isolated from the mammal (e.g., from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of FGF-CX. A monoclonal antibody composition thus typically displays a single binding affinity for a particular FGF-CX protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular FGF-CX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see Kohler & Milstein, 1975 *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983 *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Each of the above citations are incorporated herein by reference in their entirety.

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to a FGF-CX protein (see e.g., U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (see e.g., Huse, *et al.*, 1989 *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a FGF-CX protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. See e.g., U.S. Patent No. 5,225,539. Each of the above citations are incorporated herein by reference. Antibody fragments that contain the idiotypes to a FGF-CX protein may be produced by techniques known in the art including, but not limited to: (i) an $F_{(ab)2}$ fragment produced by

pepsin digestion of an antibody molecule; (ii) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab)2}$ fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

5 Additionally, recombinant anti-FGF-CX antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent
10 Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; European Patent Application No. 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.* (1987) *J Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.* (1987) *Cancer Res* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988), *J.*
15 *Natl Cancer Inst* 80:1553-1559; Morrison (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; U.S. Pat. No. 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J Immunol* 141:4053-4060. Each of the above citations are incorporated herein by reference.

20 In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of a FGF-CX protein is facilitated by generation of hybridomas that bind to the fragment of a FGF-CX protein possessing such a domain. Antibodies that are specific for one or more domains within a FGF-CX protein, *e.g.*, the
25 domain spanning the first fifty amino-terminal residues specific to FGF-CX when compared to FGF-9, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-FGF-CX antibodies may be used in methods known within the art relating to the localization and/or quantitation of a FGF-CX protein (*e.g.*, for use in measuring levels of the FGF-CX protein within appropriate physiological samples, for use in diagnostic methods, for use
30 in imaging the protein, and the like). In a given embodiment, antibodies for FGF-CX proteins, or

derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

An anti-FGF-CX antibody (*e.g.*, monoclonal antibody) can be used to isolate FGF-CX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-FGF-CX

antibody can facilitate the purification of natural FGF-CX from cells and of recombinantly produced FGF-CX expressed in host cells. Moreover, an anti-FGF-CX antibody can be used to detect FGF-CX protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the FGF-CX protein. Anti-FGF-CX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*,

to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or

acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable

radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

FGF-CX Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding FGF-CX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are

replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, FGF-CX proteins, mutant forms of FGF-CX, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of FGF-CX in prokaryotic or eukaryotic cells. For example, FGF-CX can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY:

METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

5 Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as
10 a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc;
15 Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION
20 TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein. See, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185,
25 Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

30 In another embodiment, the FGF-CX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*,

(1987) *EMBO J* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, FGF-CX can be expressed in insect cells using baculovirus expression
 5 vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith *et al.* (1983) *Mol Cell Biol* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian
 cells using a mammalian expression vector. Examples of mammalian expression vectors include
 10 pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression
 systems for both prokaryotic and eukaryotic cells. See, *e.g.*, Chapters 16 and 17 of Sambrook *et al.*,
 15 *MOLECULAR CLONING: A LABORATORY MANUAL*. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific
 20 regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell*
 25 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss (1990)
 30 *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to FGF-CX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub *et al.*, "Antisense RNA as a molecular tool for genetic analysis," Reviews--Trends in Genetics, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, FGF-CX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.*

(MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

5 For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a
10 selectable marker can be introduced into a host cell on the same vector as that encoding FGF-CX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can
15 be used to produce (*i.e.*, express) FGF-CX protein. Accordingly, the invention further provides methods for producing FGF-CX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding FGF-CX has been introduced) in a suitable medium such that FGF-CX protein is produced. In another embodiment, the method further comprises
20 isolating FGF-CX from the medium or the host cell.

Transgenic animals

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which FGF-CX-coding sequences have been introduced. Such host
25 cells can then be used to create non-human transgenic animals in which exogenous FGF-CX sequences have been introduced into their genome or homologous recombinant animals in which endogenous FGF-CX sequences have been altered. Such animals are useful for studying the function and/or activity of FGF-CX and for identifying and/or evaluating modulators of FGF-CX activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal,
30 more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep,

dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA that is integrated into the genome of a cell from which a transgenic animal develops and that remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, a "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous FGF-CX gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing FGF-CX-encoding nucleic acid into the male pronuclei of a fertilized oocyte, *e.g.*, by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The human FGF-CX DNA sequence of SEQ ID NO:1 can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human FGF-CX gene, such as a mouse FGF-CX gene, can be isolated based on hybridization to the human FGF-CX cDNA (described further above) and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the FGF-CX transgene to direct expression of FGF-CX protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Pat. Nos. 4,736,866; 4,870,009; and 4,873,191; and Hogan 1986, In: MANIPULATING THE MOUSE EMBRYO, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the FGF-CX transgene in its genome and/or expression of FGF-CX mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding FGF-CX can further be bred to other transgenic animals carrying other transgenes.

To create a homologous recombinant animal, a vector is prepared which contains at least a portion of a FGF-CX gene into which a deletion, addition or substitution has been introduced to thereby alter, *e.g.*, functionally disrupt, the FGF-CX gene. The FGF-CX gene can be a human gene (*e.g.*, SEQ ID NO:1), but more preferably, is a non-human homologue of a human FGF-

CX gene. For example, a mouse homologue of human FGF-CX gene of SEQ ID NO:1 can be used to construct a homologous recombination vector suitable for altering an endogenous FGF-CX gene in the mouse genome. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous FGF-CX gene is functionally disrupted (*i.e.*, no longer encodes a functional protein; also referred to as a "knock out" vector).

Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous FGF-CX gene is mutated or otherwise altered but still encodes functional protein (*e.g.*, the upstream regulatory region can be altered to thereby alter the expression of the endogenous FGF-CX protein). In the homologous recombination vector, the altered portion of the FGF-CX gene is flanked at its 5' and 3' ends by additional nucleic acid of the FGF-CX gene to allow for homologous recombination to occur between the exogenous FGF-CX gene carried by the vector and an endogenous FGF-CX gene in an embryonic stem cell. The additional flanking FGF-CX nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector. See *e.g.*, Thomas *et al.* (1987) *Cell* 51:503 for a description of homologous recombination vectors. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced FGF-CX gene has homologously recombined with the endogenous FGF-CX gene are selected (see *e.g.*, Li *et al.* (1992) *Cell* 69:915).

The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse) to form aggregation chimeras. See *e.g.*, Bradley 1987, In: TERATOCARCINOMAS AND EMBRYONIC STEM CELLS: A PRACTICAL APPROACH, Robertson, ed. IRL, Oxford, pp. 113-152. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Curr Opin Biotechnol* 2:823-829; PCT International Publication Nos.: WO 90/11354; WO 91/01140; WO 92/0968; and WO 93/04169.

In another embodiment, transgenic non-humans animals can be produced that contain selected systems that allow for regulated expression of the transgene. One example of such a

system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, see, *e.g.*, Lakso *et al.* (1992) *PNAS* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman *et al.* (1991) *Science* 251:1351-1355. If a cre/loxP recombinase system is used to regulate

5 expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, *e.g.*, by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced

10 according to the methods described in Wilmut *et al.* (1997) *Nature* 385:810-813. In brief, a cell, *e.g.*, a somatic cell, from the transgenic animal can be isolated and induced to exit the growth cycle and enter G₀ phase. The quiescent cell can then be fused, *e.g.*, through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or

15 blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, *e.g.*, the somatic cell, is isolated.

Pharmaceutical Compositions

The FGF-CX nucleic acid molecules, FGF-CX proteins, and anti-FGF-CX antibodies

20 (also referred to herein as "active compounds") of the invention, and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings,

25 antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, finger's solutions, dextrose solution, and 5% human

30 serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art.

Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

5 A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

15 Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable

compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a FGF-CX protein or anti-FGF-CX antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives.

Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional
5 suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid,
10 collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to
15 those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired
20 therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by any of a number of
25 routes, *e.g.*, as described in U.S. Patent Nos. 5,703,055. Delivery can thus also include, *e.g.*, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or stereotactic injection (see *e.g.*, Chen *et al.* (1994) *PNAS* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the
30 complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral

vectors, the pharmaceutical preparation can include one or more cells that produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

5 **Uses and Methods of the Invention**

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: (a) screening assays; (b) detection assays (*e.g.*, chromosomal mapping, tissue typing, forensic biology), (c) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics);
10 and (d) methods of treatment (*e.g.*, therapeutic and prophylactic). As described herein, in one embodiment, a FGF-CX protein of the invention has the ability to bind ATP.

The isolated nucleic acid molecules of the invention can be used to express FGF-CX protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect FGF-CX mRNA (*e.g.*, in a biological sample) or a genetic lesion in a FGF-CX gene, and
15 to modulate FGF-CX activity, as described further below. In addition, the FGF-CX proteins can be used to screen drugs or compounds that modulate the FGF-CX activity or expression as well as to treat disorders characterized by insufficient or excessive production of FGF-CX protein, for example proliferative or differentiative disorders, or production of FGF-CX protein forms that have decreased or aberrant activity compared to FGF-CX wild type protein. In addition, the
20 anti-FGF-CX antibodies of the invention can be used to detect and isolate FGF-CX proteins and modulate FGF-CX activity.

This invention further pertains to novel agents identified by the above described screening assays and uses thereof for treatments as described herein.

Screening Assays

25 The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, peptides, peptidomimetics, small molecules or other drugs) that bind to FGF-CX proteins or have a stimulatory or inhibitory effect on, for example, FGF-CX expression or FGF-CX activity.

In one embodiment, the invention provides assays for screening candidate or test
30 compounds which bind to or modulate the activity of a FGF-CX protein or polypeptide or

biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc Natl Acad Sci U.S.A.* 90:6909; Erb *et al.* (1994) *Proc Natl Acad Sci U.S.A.* 91:11422; Zuckermann *et al.* (1994) *J Med Chem* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2059; Carell *et al.* (1994) *Angew Chem Int Ed Engl* 33:2061; and Gallop *et al.* (1994) *J Med Chem* 37:1233.

Libraries of compounds may be presented in solution (*e.g.*, Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), on chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner U.S. Pat. No. 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla *et al.* (1990) *Proc Natl Acad Sci U.S.A.* 87:6378-6382; Felici (1991) *J Mol Biol* 222:301-310; Ladner *above.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of FGF-CX protein, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to a FGF-CX protein determined. The cell, for example, can be of mammalian origin or a yeast cell. Determining the ability of the test compound to bind to the FGF-CX protein can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the FGF-CX protein or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by

determination of conversion of an appropriate substrate to product. In one embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of FGF-CX protein, or a biologically active portion thereof, on the cell surface with a known compound which binds FGF-CX to form an assay mixture, contacting the assay mixture with a test compound, and
5 determining the ability of the test compound to interact with a FGF-CX protein, wherein determining the ability of the test compound to interact with a FGF-CX protein comprises determining the ability of the test compound to preferentially bind to FGF-CX or a biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell
10 expressing a membrane-bound form of FGF-CX protein, or a biologically active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the FGF-CX protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of FGF-CX or a biologically active portion thereof can be accomplished, for example, by determining the
15 ability of the FGF-CX protein to bind to or interact with a FGF-CX target molecule. As used herein, a "target molecule" is a molecule with which a FGF-CX protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses a FGF-CX interacting protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A
20 FGF-CX target molecule can be a non-FGF-CX molecule or a FGF-CX protein or polypeptide of the present invention. In one embodiment, a FGF-CX target molecule is a component of a signal transduction pathway that facilitates transduction of an extracellular signal (*e.g.*, a signal generated by binding of a compound to a membrane-bound FGF-CX molecule) through the cell membrane and into the cell. The target, for example, can be a second intercellular protein that
25 has catalytic activity or a protein that facilitates the association of downstream signaling molecules with FGF-CX.

Determining the ability of the FGF-CX protein to bind to or interact with a FGF-CX target molecule can be accomplished by one of the methods described above for determining direct binding. In one embodiment, determining the ability of the FGF-CX protein to bind to or
30 interact with a FGF-CX target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting

induction of a cellular second messenger of the target (*i.e.* intracellular Ca^{2+} , diacylglycerol, IP_3 , etc.), detecting catalytic/enzymatic activity of the target an appropriate substrate, detecting the induction of a reporter gene (comprising a FGF-CX-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular
5 response, for example, cell survival, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a FGF-CX protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the FGF-CX protein or biologically active portion thereof. Binding of the test compound to the FGF-CX protein can be
10 determined either directly or indirectly as described above. In one embodiment, the assay comprises contacting the FGF-CX protein or biologically active portion thereof with a known compound which binds FGF-CX to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a FGF-CX protein, wherein determining the ability of the test compound to interact with a FGF-CX protein
15 comprises determining the ability of the test compound to preferentially bind to FGF-CX or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting FGF-CX protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (*e.g.*, stimulate or inhibit) the activity of the FGF-CX protein or
20 biologically active portion thereof. Determining the ability of the test compound to modulate the activity of FGF-CX can be accomplished, for example, by determining the ability of the FGF-CX protein to bind to a FGF-CX target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of FGF-CX can be accomplished by determining the ability
25 of the FGF-CX protein further modulate a FGF-CX target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the FGF-CX protein or biologically active portion thereof with a known compound which binds FGF-CX to form an
30 assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a FGF-CX protein, wherein determining the ability of the test

compound to interact with a FGF-CX protein comprises determining the ability of the FGF-CX protein to preferentially bind to or modulate the activity of a FGF-CX target molecule.

The cell-free assays of the present invention are amenable to use of both the soluble form or the membrane-bound form of FGF-CX. In the case of cell-free assays comprising the membrane-bound form of FGF-CX, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of FGF-CX is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, N-dodecyl--N,N-dimethyl-3-ammonio-1-propane sulfonate, 3-(3-cholamidopropyl)dimethylamminiol-1-propane sulfonate (CHAPS), or 3-(3-cholamidopropyl)dimethylamminiol-2-hydroxy-1-propane sulfonate (CHAPSO).

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either FGF-CX or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to FGF-CX, or interaction of FGF-CX with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided that adds a domain that allows one or both of the proteins to be bound to a matrix. For example, GST-FGF-CX fusion proteins or GST-target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, that are then combined with the test compound or the test compound and either the non-adsorbed target protein or FGF-CX protein, and the mixture is incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of FGF-CX binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either FGF-CX or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated FGF-CX or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with FGF-CX or target molecules, but which do not interfere with binding of the FGF-CX protein to its target molecule, can be derivatized to the wells of the plate, and unbound target or FGF-CX trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the FGF-CX or target molecule, as well as enzyme-linked assays that rely on detecting an enzymatic activity associated with the FGF-CX or target molecule.

In another embodiment, modulators of FGF-CX expression are identified in a method wherein a cell is contacted with a candidate compound and the expression of FGF-CX mRNA or protein in the cell is determined. The level of expression of FGF-CX mRNA or protein in the presence of the candidate compound is compared to the level of expression of FGF-CX mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of FGF-CX expression based on this comparison. For example, when expression of FGF-CX mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of FGF-CX mRNA or protein expression. Alternatively, when expression of FGF-CX mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of FGF-CX mRNA or protein expression. The level of FGF-CX mRNA or protein expression in the cells can be determined by methods described herein for detecting FGF-CX mRNA or protein.

In yet another aspect of the invention, the FGF-CX proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, e.g., U.S. Pat. No. 5,283,317; Zervos *et al.* (1993) Cell 72:223-232; Madura *et al.* (1993) J Biol Chem 268:12046-12054; Bartel *et al.* (1993) Biotechniques 14:920-924; Iwabuchi *et al.* (1993) Oncogene 8:1693-1696; and Brent WO94/10300), to identify other proteins that bind to or interact with FGF-CX ("FGF-

CX-binding proteins" or "FGF-CX-bp") and modulate FGF-CX activity. Such FGF-CX-binding proteins are also likely to be involved in the propagation of signals by the FGF-CX proteins as, for example, upstream or downstream elements of the FGF-CX pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for FGF-CX is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a FGF-CX-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) that is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene that encodes the protein which interacts with FGF-CX.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome; and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample.

The FGF-CX sequences of the present invention can also be used to identify individuals from minute biological samples. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP ("restriction fragment length polymorphisms," described in U.S. Pat. No. 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique that determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the FGF-CX sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to
5 amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The FGF-CX sequences
10 of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Much of the allelic variation is due to single nucleotide polymorphisms (SNPs), which include restriction fragment length polymorphisms (RFLPs).

Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1, as described above, can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000
15 primers that each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences are used, a more appropriate number of primers for positive individual identification would be 500-2,000.
20

Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic
25 assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining FGF-CX protein and/or nucleic acid expression as well as FGF-CX activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a
30 disease or disorder, or is at risk of developing a disorder, associated with aberrant FGF-CX expression or activity. The invention also provides for prognostic (or predictive) assays for

determining whether an individual is at risk of developing a disorder associated with FGF-CX protein, nucleic acid expression or activity. For example, mutations in a FGF-CX gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with FGF-CX protein, nucleic acid expression or activity.

Another aspect of the invention provides methods for determining FGF-CX protein, nucleic acid expression or FGF-CX activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (*e.g.*, drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (*e.g.*, the genotype of the individual examined to determine the ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of FGF-CX in clinical trials.

These and other agents are described in further detail in the following sections.

Diagnostic Assays

Fibroblast growth factors FGF-1 through FGF-9 generally promote cell proliferation in cells carrying the particular growth factor receptor. Examples of FGF growth promotion include epithelial cells, such as fibroblasts and keratinocytes, in the anterior eye after surgery. Other conditions in which proliferation of cells plays a role include tumors, restenosis, psoriasis, Dupuytren's contracture, diabetic complications, Kaposi's sarcoma and rheumatoid arthritis.

FGF-CX may be used in the method of the invention for detecting its corresponding fibroblast growth factor receptor CX (FGFRCX) in a sample or tissue. The method comprises contacting the sample or tissue with FGF-CX, allowing formation of receptor-ligand pairs, and detecting any FGFRCX: FGF-CX pairs. Compositions containing FGF-CX can be used to increase FGFRCX activity, for example to stimulate cartilage or bone repair. Compositions containing FGF-CX antagonists or FGF-CX binding agents (*e.g.* anti- FGF-CX antibodies) can be used to treat diseases caused by an excess of FGF-CX or overactivity of FGFRCX, especially multiple or solitary hereditary exostosis, hallux valgus deformity, achondroplasia, synovial chondromatosis and endochondromas.

Glia activating factor (GAF) and the DNA encoding GAF act to specifically promote growth of glial cells. Some examples of glia-associated disorders in which GAF may be utilized to modulate glial cell activities are cerebral lesions, cerebral edema, senile dementia, Alzheimer's disease, diabetic neuropathies, etc. Similarly, FGF-CX may be used in diagnosis or treating glial cell related disorders. The glial-cell modulating activity of FGF-CX may be as a neuroprotective-like activity, and FGF-CX may be used as a neuroprotective agent. Due to the close homology of FGF-CX to FGF-9, which was identified originally as a glia activating factor, it can be presumed that the FGF-CX sequence is also a glia activating factor. FGF-CX can therefore be used to stimulate the growth of glia cells and can be used to accelerate healing of cerebral lesions or to treat cerebral edema, senile dementia, Alzheimer's disease, or diabetic neuropathy.

FGF-CX can also be used to stimulates fibroblasts (for accelerating healing of burns, wounds, ulcers, etc), megakaryocytes (to increase the number of platelets), hematopoietic cells, immune system cells, and vascular smooth muscle cells. FGF-CX is also expected to have osteogenesis-promoting activity, and can be used for treating bone fractures and osteoporosis. Assay of FGF-CX polypeptide or nucleic acid moieties may be useful in diagnosis of cerebral tumors, and antibodies against could be used to treat such tumors. It can also be used as a reagent for stimulating growth of cultured cells. An anticipated dosage is 1ng-0.1mg/kg/day, though treatment may vary depending on the type or severity of the disorder being treated. FGF-CX polypeptides may be used as platelet increasing agents, osteogenesis promoting agents or for treating cerebral nervous diseases or hepatopathy such as hepatic cirrhosis. They can also be used to treat cancer when used alongside an anticancer agent. Antibodies directed against the FGF-CX polypeptide, or fragments, derivatives, or analogs thereof, can be used for detecting or determining a biological activity of a FGF-CX polypeptide or for purifying a FGF-CX polypeptide. Those antibodies that also neutralize the cell growth activity of FGF-CX can be used as anticancer agents.

Many, if not all, homologous proteins are known in the art to have closely related or identical functions. See, *e.g.*, Lewin, "Chapter 21: Structural Genes Belong to Families" In: GENES II, 1985, John Wiley and Sons, Inc., New York. The FGF-CX polypeptide closely resembles the Xenopus XFGF-20 protein, which was shown previously to be specifically expressed in highly proliferative tissues (see, *e.g.*, Koga *et al.*, above). Therefore, it is presumed that FGF-CX would also modulate cellular activity in highly proliferative tissues. FGF-CX may

thus be particularly useful in diagnosing proliferative disorders and in stimulating the growth of cells and tissues in order to overcome pathological states in which such growth has been suppressed or inhibited. Oligonucleotides corresponding to any one portion of the FGF-CX nucleic acids of SEQ ID NO:1 may be used to detect the expression of a FGF-CX-like gene. The proteins of the invention may be used to stimulate production of antibodies specifically binding the proteins. Such antibodies may be used in immunodiagnostic procedures to detect the occurrence of the protein in a sample. The proteins of the invention may be used to stimulate cell growth and cell proliferation in conditions in which such growth would be favorable. An example would be to counteract toxic side effects of chemotherapeutic agents on, for example, hematopoiesis and platelet formation, linings of the gastrointestinal tract, and hair follicles. They may also be used to stimulate new cell growth in neurological disorders including, for example, Alzheimer's disease. Alternatively, antagonistic treatments may be administered in which an antibody specifically binding the FGF-CX-like proteins of the invention would abrogate the specific growth-inducing effects of the proteins. Such antibodies may be useful, for example, in the treatment of proliferative disorders including various tumors and benign hyperplasias.

An exemplary method for detecting the presence or absence of FGF-CX in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting FGF-CX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes FGF-CX protein such that the presence of FGF-CX is detected in the biological sample. An agent for detecting FGF-CX mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to FGF-CX mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length FGF-CX nucleic acid, such as the nucleic acid of SEQ ID NO:1, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to FGF-CX mRNA or genomic DNA, as described above. Other suitable probes for use in the diagnostic assays of the invention are described herein.

An agent for detecting FGF-CX protein is an antibody capable of binding to FGF-CX protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to

the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect FGF-CX mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of FGF-CX mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of FGF-CX protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of FGF-CX genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of FGF-CX protein include introducing into a subject a labeled anti-FGF-CX antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting FGF-CX protein, mRNA, or genomic DNA, such that the presence of FGF-CX protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of FGF-CX protein, mRNA or genomic DNA in the control sample with the presence of FGF-CX protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of FGF-CX in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting FGF-CX protein or mRNA in a biological sample; means for determining the amount of FGF-CX in the sample; and means for comparing the amount of FGF-CX in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect FGF-CX protein or nucleic acid.

Prognostic Assays

The diagnostic methods described herein can furthermore be utilized to identify subjects having or at risk of developing a disease or disorder associated with aberrant FGF-CX expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with FGF-CX protein, nucleic acid expression or activity in, *e.g.*, proliferative or differentiative disorders such as hyperplasias, tumors, restenosis, psoriasis, Dupuytren's contracture, diabetic complications, or rheumatoid arthritis, etc.; and glia-associated disorders such as cerebral lesions, diabetic neuropathies, cerebral edema, senile dementia, Alzheimer's disease, etc. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing a disease or disorder. Thus, the present invention provides a method for identifying a disease or disorder associated with aberrant FGF-CX expression or activity in which a test sample is obtained from a subject and FGF-CX protein or nucleic acid (*e.g.*, mRNA, genomic DNA) is detected, wherein the presence of FGF-CX protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant FGF-CX expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (*e.g.*, serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant FGF-CX expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent for a disorder, such as a proliferative disorder, differentiative disorder, glia-associated disorders, etc. Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant FGF-CX expression or activity in which a test sample is obtained and FGF-CX protein or nucleic acid is detected (*e.g.*, wherein the presence of FGF-CX protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant FGF-CX expression or activity.)

The methods of the invention can also be used to detect genetic lesions in a FGF-CX gene, thereby determining if a subject with the lesioned gene is at risk for, or suffers from, a

proliferative disorder, differentiative disorder, glia-associated disorder, etc. In various embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion characterized by at least one of an alteration affecting the integrity of a gene encoding a FGF-CX-protein, or the mis-expression of the FGF-CX gene. For example, such genetic lesions can be detected by ascertaining the existence of at least one of (1) a deletion of one or more nucleotides from a FGF-CX gene; (2) an addition of one or more nucleotides to a FGF-CX gene; (3) a substitution of one or more nucleotides of a FGF-CX gene, (4) a chromosomal rearrangement of a FGF-CX gene; (5) an alteration in the level of a messenger RNA transcript of a FGF-CX gene, (6) aberrant modification of a FGF-CX gene, such as of the methylation pattern of the genomic DNA, (7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a FGF-CX gene, (8) a non-wild type level of a FGF-CX-protein, (9) allelic loss of a FGF-CX gene, and (10) inappropriate post-translational modification of a FGF-CX-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in a FGF-CX gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, *e.g.*, U.S. Pat. Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, *e.g.*, Landegran *et al.* (1988) *Science* 241:1077-1080; and Nakazawa *et al.* (1994) *PNAS* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the FGF-CX-gene (see Abravaya *et al.* (1995) *Nucl Acids Res* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers that specifically hybridize to a FGF-CX gene under conditions such that hybridization and amplification of the FGF-CX gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli *et al.*, 1990, *Proc Natl Acad Sci USA* 87:1874-1878), transcriptional amplification system (Kwoh, *et al.*, 1989, *Proc Natl Acad Sci USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.*, 1988, *BioTechnology* 6:1197), or any other nucleic acid amplification method. followed by the
 5 detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a FGF-CX gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and
 10 control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Pat. No. 5,493,531) can be used to score for the presence of specific mutations by development or loss of
 15 a ribozyme cleavage site.

In other embodiments, genetic mutations in FGF-CX can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin *et al.* (1996) *Human Mutation* 7: 244-255; Kozal *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in FGF-CX
 20 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin *et al.* above. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the
 25 characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the FGF-CX gene and detect mutations by comparing the sequence
 30 of the sample FGF-CX with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert (1977)

PNAS 74:560 or Sanger (1977) *PNAS* 74:5463. It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve *et al.*, (1995) *Biotechniques* 19:448), including sequencing by mass spectrometry (see, *e.g.*, PCT International Publ. No. WO 94/16101; Cohen *et al.* (1996) *Adv Chromatogr* 36:127-162; and Griffin *et al.* (1993) *Appl Biochem Biotechnol* 38:147-159).

Other methods for detecting mutations in the FGF-CX gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242). In general, the art technique of "mismatch cleavage" starts by providing heteroduplexes of formed by hybridizing (labeled) RNA or DNA containing the wild-type FGF-CX sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent that cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA hybrids treated with S1 nuclease to enzymatically digesting the mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, for example, Cotton *et al* (1988) *Proc Natl Acad Sci USA* 85:4397; Saleeba *et al* (1992) *Methods Enzymol* 217:286-295. In an embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in FGF-CX cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a FGF-CX sequence, *e.g.*, a wild-type FGF-CX sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, for example, U.S. Pat. No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in FGF-CX genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl Acad Sci USA*: 86:2766, see also Cotton (1993) *Mutat Res* 285:125-144; Hayashi (1992) *Genet Anal Tech Appl* 9:73-79). Single-stranded DNA fragments of sample and control FGF-CX nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA, rather than DNA, in which the secondary structure is more sensitive to a change in sequence. In one embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility. See, *e.g.*, Keen *et al.* (1991) *Trends Genet* 7:5.

In yet another embodiment the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE). See, *e.g.*, Myers *et al.* (1985) *Nature* 313:495. When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA. See, *e.g.*, Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753.

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions that permit hybridization only if a perfect match is found. See, *e.g.*, Saiki *et al.* (1986) *Nature* 324:163; Saiki *et al.* (1989) *Proc Natl Acad. Sci USA* 86:6230. Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology that depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as

primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection. See, *e.g.*, Gasparini *et al* (1992) *Mol Cell Probes* 6:1. It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification. See, *e.g.*, Barany (1991) *Proc Natl Acad Sci USA* 88:189. In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence, making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a FGF-CX gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which FGF-CX is expressed may be utilized in the prognostic assays described herein. However, any biological sample containing nucleated cells may be used, including, for example, buccal mucosal cells.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on FGF-CX activity (*e.g.*, FGF-CX gene expression), as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (*e.g.*, neurological, cancer-related or gestational disorders) associated with aberrant FGF-CX activity. In conjunction with such treatment, the pharmacogenomics (*i.e.*, the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (*e.g.*, drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can

further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of FGF-CX protein, expression of FGF-CX nucleic acid, or mutation content of FGF-CX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

5 Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See *e.g.*, Eichelbaum, 1996, *Clin Exp Pharmacol Physiol*, 23:983-985 and Linder, 1997, *Clin Chem*, 43:254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug
10 action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and
15 consumption of fava beans.

 As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (*e.g.*, N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why
20 some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified
25 in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, PM show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not
30 respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of FGF-CX protein, expression of FGF-CX nucleic acid, or mutation content of FGF-CX genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a FGF-CX modulator, such as a modulator identified by one of the exemplary screening assays described herein.

Monitoring Clinical Efficacy

Monitoring the influence of agents (*e.g.*, drugs, compounds) on the expression or activity of FGF-CX (*e.g.*, the ability to modulate aberrant cell proliferation and/or differentiation) can be applied in basic drug screening and in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase FGF-CX gene expression, protein levels, or upregulate FGF-CX activity, can be monitored in clinical trials of subjects exhibiting decreased FGF-CX gene expression, protein levels, or downregulated FGF-CX activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease FGF-CX gene expression, protein levels, or downregulate FGF-CX activity, can be monitored in clinical trials of subjects exhibiting increased FGF-CX gene expression, protein levels, or upregulated FGF-CX activity. In such clinical trials, the expression or activity of FGF-CX and, preferably, other genes that have been implicated in, for example, a proliferative or neurological disorder, can be used as a "read out" or marker of the responsiveness of a particular cell.

For example, genes, including FGF-CX, that are modulated in cells by treatment with an agent (*e.g.*, compound, drug or small molecule) that modulates FGF-CX activity (*e.g.*, identified in a screening assay as described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of FGF-CX and other genes implicated in the disorder. The levels of gene expression (*i.e.*, a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of FGF-CX or other genes. In this way, the gene expression pattern can serve as a

marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In one embodiment, the invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, an agonist, antagonist, protein, peptide, nucleic acid, peptidomimetic, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a FGF-CX protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the FGF-CX protein, mRNA, or genomic DNA in the post-administration samples; (v) comparing the level of expression or activity of the FGF-CX protein, mRNA, or genomic DNA in the pre-administration sample with the FGF-CX protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of FGF-CX to higher levels than detected, *i.e.*, to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of FGF-CX to lower levels than detected, *i.e.*, to decrease the effectiveness of the agent.

Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant FGF-CX expression or activity.

Diseases and disorders that are characterized by increased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) activity. Therapeutics that antagonize activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, (i) a FGF-CX polypeptide, or analogs, derivatives, fragments or homologs thereof; (ii) antibodies to a FGF-CX peptide; (iii) nucleic acids encoding a FGF-CX peptide; (iv) administration of antisense nucleic acid and nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding

sequences to a FGF-CX peptide) that are utilized to "knockout" endogenous function of a FGF-CX peptide by homologous recombination (see, *e.g.*, Capecchi, 1989, *Science* 244: 1288-1292); or (v) modulators (*i.e.*, inhibitors, agonists and antagonists, including additional peptide mimetic of the invention or antibodies specific to a peptide of the invention) that alter the interaction between a FGF-CX peptide and its binding partner.

Diseases and disorders that are characterized by decreased (relative to a subject not suffering from the disease or disorder) levels or biological activity may be treated with Therapeutics that increase (*i.e.*, are agonists to) activity. Therapeutics that upregulate activity may be administered in a therapeutic or prophylactic manner. Therapeutics that may be utilized include, but are not limited to, a FGF-CX peptide, or analogs, derivatives, fragments or homologs thereof; or an agonist that increases bioavailability.

Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or peptide levels, structure and/or activity of the expressed peptides (or mRNAs of a FGF-CX peptide). Methods that are well-known within the art include, but are not limited to, 5 immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.).

In one aspect, the invention provides a method for preventing, in a subject, a disease or 10 condition associated with an aberrant FGF-CX expression or activity, by administering to the subject an agent that modulates FGF-CX expression or at least one FGF-CX activity. Subjects at risk for a disease that is caused or contributed to by aberrant FGF-CX expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of 15 symptoms characteristic of the FGF-CX aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of FGF-CX aberrancy, for example, a FGF-CX agonist or FGF-CX antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

Another aspect of the invention pertains to methods of modulating FGF-CX expression or 20 activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of FGF-CX protein activity associated with the cell. An agent that modulates FGF-CX protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of a FGF-CX protein, a peptide, a FGF-CX peptidomimetic, or other small molecule. In one 25 embodiment, the agent stimulates one or more FGF-CX protein activity. Examples of such stimulatory agents include active FGF-CX protein and a nucleic acid molecule encoding FGF-CX that has been introduced into the cell. In another embodiment, the agent inhibits one or more FGF-CX protein activity. Examples of such inhibitory agents include antisense FGF-CX nucleic acid molecules and anti-FGF-CX antibodies. These modulatory methods can be performed *in* 30 *vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering

the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a FGF-CX protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) FGF-CX expression or activity. In another embodiment, the method involves administering a FGF-CX protein or nucleic acid molecule as therapy to compensate for reduced or aberrant FGF-CX expression or activity.

EXAMPLES

Example 1. Molecular Cloning of the Sequence Encoding a FGF-CX Protein.

Oligonucleotide primers were designed for the amplification by PCR of a DNA segment, representing an open reading frame, coding for the full length FGF-CX. The forward primer includes a BglII restriction site (AGATCT) and a consensus Kozak sequence (CCACC). The reverse primer contains an in-frame XhoI restriction site for further subcloning purposes. Both the forward and the reverse primers contain a 5' clamp sequence (CTCGTC). The sequences of the primers are the following:

FGF-CX-Forward: 5' - CTCGTC AGATCT CCACC ATG GCT CCC TTA GCC GAA GTC - 3' (SEQ ID NO:3)

FGF-CX-Reverse: 5' - CTCGTC CTCGAG AGT GTA CAT CAG TAG GTC CTT G - 3' (SEQ ID NO:4)

PCR reactions were set up using a total of 5ng human prostate cDNA template, 1 μ M of each of the FGF-CX-Forward and FGF-CX-Reverse primers, 5 micromoles dNTP (Clontech Laboratories, Palo Alto CA) and 1 microliter of 50xAdvantage-HF 2 polymerase (Clontech Laboratories, Palo Alto CA) in 50 microliter volume. The following PCR reaction conditions were used:

- a) 96°C 3 minutes
- b) 96°C 30 seconds denaturation
- c) 70°C 30 seconds, primer annealing. This temperature was gradually decreased by 1°C/cycle.

d) 72°C 1 minute extension.

Repeat steps (b)-(d) ten times

e) 96°C 30 seconds denaturation

f) 60°C 30 seconds annealing

5 g) 72°C 1 minute extension

Repeat steps (e)-(g) 25 times

h) 72°C 5 minutes final extension

A single PCR product, with the expected size of approximately 640 bp, was isolated from agarose gel and ligated into a pCR2.1 vector (Invitrogen, Carlsbad, CA). The cloned insert was
10 sequenced using vector specific M13 Forward(-40) and M13 Reverse primers, which verified that the nucleotide sequence was 100% identical to the sequence in FIG. 1 (SEQ ID NO:1) inserted directly between the upstream BglII cloning site and the downstream XhoI cloning site. The cloned sequence constitutes an open reading frame coding for the predicted cgAB02085 full length protein. The clone is called TA- cgAB02085-S274-F19.

15 All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EQUIVALENTS

20 From the foregoing detailed description of the specific embodiments of the invention, it should be apparent that particular novel compositions and methods involving nucleic acids, polypeptides, antibodies, detection and treatment have been described. Although these particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the
25 appended claims that follow. In particular, it is contemplated by the inventors that various substitutions, alterations, and modifications may be made as a matter of routine for a person of ordinary skill in the art to the invention without departing from the spirit and scope of the invention as defined by the claims. Indeed, various modifications of the invention in addition to

those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.